

CHAPTER 23

NEOGEN REVEAL FOR AFLATOXIN SQ TEST KIT

Product Number 8020

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23.1 GENERAL INFORMATION

The Neogen Reveal for Aflatoxin SQ test kit (product number 8020) is a single-step lateral flow immunochromatographic assay based on a competitive immunoassay format. The test provides a qualitative determination of the presence or absence of aflatoxin at a 10 ppb threshold level in corn only. Using an aflatoxin-antibody particle complex coated test strip, the inspector is able to determine the concentration of aflatoxin by the density of a test line. Therefore, as the concentration of aflatoxin in the sample increases, the test line density decreases. Algorithms programmed into the Neogen Reveal AccuScan III reader convert these line densities into a semi-quantitative result displayed in parts per billion (ppb).

23.2 PREPARATION OF EXTRACTION SOLUTION

The extraction solvent used in the Neogen Reveal for Aflatoxin SQ test method is a methanol/water (distilled or deionized) mixture consisting of 70 percent methanol (Reagent grade or better) and 30 percent water.

- a. Using a graduated cylinder, measure 700 ml of methanol and place it into a clean carboy with spigot.
- b. Add 300 ml deionized or distilled water to the methanol and shake vigorously until it is completely mixed.
- c. Label the container stating the mixture (70 percent methanol and 30 percent water), date of preparation, and initials of technician who prepared the solution.
- d. Store this solution at room temperature in a tightly closed container until needed.

NOTE: To prepare smaller or larger amounts of solution use the ratio of 7 parts methanol to 3 parts of deionized or distilled water.

23.3 EXTRACTION PROCEDURES

- a. Transfer 50 grams of ground sample into an extraction mixing jar.
- b. Add 250 ml of the (70/30) methanol/water extraction solvent.
- c. Cover the extraction jar and blend on high speed for 1 minute or shake vigorously for 3 minutes.

- d. Allow the sample to settle, filter the extract through a filter syringe or filter paper, and place into a sample jar labeled with the sample identification.
- e. After collecting the filtrate dispose of the filter and ground material and set the filtrate aside for testing.

23.4 TEST PROCEDURES

a. Sample Analysis.

- (1) Remove the appropriate number of sample cups and place in a sample cup rack.
- (2) Using a pipettor and new tip, add 200 microliters (μl) of sample diluent to a sample cup.
- (3) Using a new tip, add 200 μl of the clear portion of the filtered sample extract to the sample cup. (This is a 1:1 dilution.)
- (4) Mix the solution by pipetting up and down three times.
- (5) Place a new Aflatoxin SQ test strip with the sample end down into the sample cup.
- (6) Allow the strip to develop in the sample cup for 5 minutes.
- (7) At the end of the 5 minute development period, remove the strip from the sample cup and read the results with Neogen's Reveal AccuScan III Reader.
- (8) Test strips should be read within 1 minute of completion of the 5 minute incubation.

b. Reading Lateral Flow Test Strip Results with the Neogen Reveal AccuScan III Reader.

The strips can only be read using Neogen's Reveal AccuScan III Reader that incorporates a Personal Data Assistant (PDA) with a Reveal AccuScan Lateral Flow optical reader.

The Neogen Reveal AccuScan III Reader will report the following value based on the results

Negative ≤ 10 ppb.

Positive > 10 ppb.

Review the Neogen Reveal AccuScan III Reader user guide for proper set-up and operating instructions before official testing.

23.5 REPORTING AND CERTIFYING TEST RESULTS

- a. Report results on the pan ticket and inspection log as being equal to or less than 10 ppb (≤ 10 ppb), or as exceeding 10 ppb (> 10 ppb), as applicable.
- b. Certify results as being equal to or less than 10 ppb or exceeding 10 ppb, as applicable.
- c. Refer to the Certification section of the handbook for more detailed certification procedures.

23.6 CLEANING LABWARE

- a. Negative Tests (≤ 10 ppb).

- (1) Labware.

Prepare a solution consisting of dishwashing liquid and water. Completely submerge the used extraction mixing jars, wash thoroughly, then rinse with clean water before reusing.

- (2) Disposable Materials.

Place materials in a garbage bag for routine trash disposal.

b. Positive Tests (> 10 ppb).

(1) Labware.

If the result is positive (more than 10 ppb) the test sample is considered hazardous. Prepare a bleach solution consisting of 1 part bleach to 10 parts water (e.g., 100 ml bleach to 1,000 ml water). Completely submerge the used extraction mixing jars and soak for at least 5 minutes. Remove items from the bleach/water solution, submerge in a dishwashing liquid/water solution, wash thoroughly, then rinse with clean water before reusing.

(2) Disposable Materials.

Prepare a bleach solution consisting of 1 part bleach to 10 parts water in a plastic pail labeled "bleach solution". Soak disposable materials, such as used test strips and pipettes for at least 5 minutes. Pour off the liquid down the drain and place the materials in a garbage bag and discard.

23.7 WASTE DISPOSAL

a. Negative Results (\leq 10 ppb).

If the test result is negative (equal to or less than 10 ppb) dispose any remaining liquid filtrate into the chemical waste container. Discard the sample slurry (ground material) into a plastic garbage bag for disposal.

b. Positive Results (> 10 ppb).

If the result is positive (more than 10 ppb) the test sample is considered hazardous. The slurry (ground portion) remaining in the sample extraction jar must be decontaminated prior to disposal. After disposing of the remaining filtered extract in the chemical waste container, pour approximately 50 ml of bleach solution into the sample extraction jar and shake to mix with the sample slurry. After the slurry and bleach solution separate, handle the bleach rinse filtrate as a non-hazardous solution and dispose of by pouring the liquid down the drain. Discard the sample slurry (ground portion) paper into a plastic garbage bag for disposal.

23.8 EQUIPMENT AND SUPPLIES

- a. Materials Supplied in Test Kits.
- (1) 25 aflatoxin SQ test strips (product # 8020).
 - (2) 25 sample cups.
 - (3) 1 bottle of sample diluent.
- b. Materials Required but not Provided.
- (1) Neogen Reveal AccuScan III Reader.
 - (2) Timer (5 minute capacity).
 - (3) 200 µl pipettor (single or multi-channel) with tips.
 - (4) Sample collection cups.
 - (5) Balance.
 - (6) Sample Grinder.
 - (7) Methanol - ACS grade or better.
 - (8) Deionized or Distilled Water.
 - (9) Blender with mixing jars.
 - (10) Filter Paper or Coffee Filters.

23.9 STORAGE CONDITIONS

- a. Storage Conditions.
- (1) Store kit components at room temperature (18°- 30°C, 64°- 86°F) at room temperature when not in use to assure full shelf life. Test strips must remain capped in the stay-dry tube before use to assure optimal performance.

(2) Do not freeze test kit components.

b. Precautions.

(1) Do not use kit components beyond expiration date.

(2) Test strip development times other than those specified may give inaccurate results.